

## PATENT COOPERATION TREATY

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ETATS-UNIS D'AMERIQUENOTIFICATION OF TRANSMITTAL OF  
THE INTERNATIONAL PRELIMINARY  
EXAMINATION REPORT  
(PCT Rule 71.1)Date of mailing  
(day/month/year)

27. 04. 98

Applicant's or agent's file reference

07257/090W01 07257/041W01

## IMPORTANT NOTIFICATION

International application No.  
PCT/US97/01457International filing date (day/month/year)  
31/01/1997Priority date (day/month/year)  
31/01/1996

Applicant

THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.

1. The applicant is hereby notified that this International Preliminary Examining Authority transmits herewith the international preliminary examination report and its annexes, if any, established on the international application.
2. A copy of the report and its annexes, if any, is being transmitted to the International Bureau for communication to all the elected Offices.
3. Where required by any of the elected Offices, the International Bureau will prepare an English translation of the report (but not of any annexes) and will transmit such translation to those Offices.

## 4. REMINDER


The applicant must enter the national phase before each elected Office by performing certain acts (filing translations and paying national fees) within 30 months from the priority date (or later in some Offices) (Article 39(1)) (see also the reminder sent by the International Bureau with Form PCT/IB/301).

Where a translation of the international application must be furnished to an elected Office, that translation must contain a translation of any annexes to the international preliminary examination report. It is the applicant's responsibility to prepare and furnish such translation directly to each elected Office concerned.

For further details on the applicable time limits and requirements of the elected Offices, see Volume II of the PCT Applicant's Guide.

* No Docketing Required *
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# PATENT COOPERATION TREATY

## PCT

### INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)



Applicant's or agent's file reference 07257/030WO1	<b>FOR FURTHER ACTION</b>		See Notification of Transmittal of International Preliminary Examination Report (PCT/IPEA/416)
International application No. PCT/US97/01457	International filing date (day/month/year) 31/01/1997	Priority date (day/month/year) 31/01/1996	
International Patent Classification (IPC) or national classification and IPC C12N15/12			
Applicant THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.			

- This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.
- This REPORT consists of a total of 7 sheets, including this cover sheet.
  - ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of    sheets.

- This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☐ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand  29/08/1997	Date of completion of this report  27. 04. 98
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# INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/US97/01457

## I. Basis of the report

1. This report has been drawn on the basis of (*substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.*):

### Description, pages:

1-62 as originally filed

### Claims, No.:

1-56 as originally filed

### Drawings, sheets:

1/10-10/10 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

## II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
  - ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

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Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

3. Additional observations, if necessary:

**see separate sheet**

**V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Yes:	Claims	3, 9-10, 12, 14-45, 48-56
	No:	Claims	1-2, 4-8, 11, 13, 46-47
Inventive step (IS)	Yes:	Claims	
	No:	Claims	1-56
Industrial applicability (IA)	Yes:	Claims	1-56
	No:	Claims	

2. Citations and explanations

**see separate sheet**

**VIII. Certain observations on the international application**

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

**see separate sheet**

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1. This international preliminary examination report (IPER) has been done considering the priority date 31.01.96 as a valid date. If it was not so the documents : (a) R. Heim and R.Y. Tsien, Current Biology 1996, Vol. 6(2), pages 178-182 and (b) R.D. Mitra et al., Gene 1996, Vol. 173(1), pages 13-17, would become relevant.

2. The following documents have been cited in the International Search Report (ISR) and have been found to be relevant for assessing the novelty and inventiveness of the claimed subject matter :

a) A.B. Cubitt et al., TIBS Trends in Biochemical Sciences 1995, Vol. 20, pages 448-455 (**D1**). On page 454 of this document reference is made to the use of fluorescence resonance energy transfer (FRET) for monitoring protein-protein interactions and in particular the authors state that "...FRET does occur between Y66H and S65C when fused together via a cleavable 25-residue spacer...". Furthermore a clear indication is also given to the skilled person for trying additional and more advantageous fusion products similar to the disclosed one ("...Ultimately, one would prefer to avoid UV excitation and use something like S65T as a donor and a green-absorbing, yellow- or red-emitting mutant as the acceptor ...") (see also Figure 4 on page 454 of **D1**). The IPEA considers that **D1** clearly discloses the subject matter of claims 1-2, 4-8, 11 and 13, which thus does not fulfil the requirements of Articles 33 (2) and (3) PCT.

b) In view of the prior art cited in the ISR concerning FRET and its applications : WO-A-94/28166 (**D2**, use of FRET substrates in assays for compound screening, identification of modulators of protease activity), C. Graham Knight Methods in Enzymology 1995, Vol. 248, pages 18-34 (**D3**, use of FRET for screening synthetic peptide libraries), K.F. Geoghegan et al., Bioconjugate Chemistry 1993, Vol. 4, pages 537-544 (**D4**, use of FRET for following a protease hydrolytic reaction and studying protease specificity), etc.. and in particular document **D5** (WO-A-91/01305) which refers to the advantageous production of modified bioluminescent proteins or "rainbow proteins" by recombinant DNA technology and their use in the detection, location, measurement or visualization of substances within or outside microbes, whole tissues, whole organisms, cells or biological molecules (pages 9-10 and claim 15), the IPEA considers that the subject matter of claims relating to :

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(1) the production of the tandem fluorescent protein construct disclosed in **D1** by recombinant DNA technology (recombinant nucleic acids, expression vectors, host cells, etc..) does not require an inventive contribution (references to Aequorea GFP DNA sequences and recombinant production of Aequorea GFP are also found in **D1**, on page 454 explicitly compares the advantages of FRET with the "two-hybrid system" , a system which has been mainly used with recombinant DNA technology).

(2) it does not require any inventiveness to use the tandem fluorescent protein constructs disclosed in **D1** in the same methods and/or for the same applications than for other known FRET substrates, and

(3) the substitution of the specific components of the tandem fluorescent protein constructs disclosed in **D1** by other arbitrary (no evident advantage) or alternative products (easily achievable by the skilled person) (P4-3, W7, etc...cleavage recognition site for HIV-1 protease, enterokinase, b-lactamase, etc..) does not involve any inventive step.

Thus, the IPEA considers that the subject matter of claims 3, 9-10, 12 and 14-45 does not fulfil the requirements of Article 33 (3) PCT.

c) document WO-A-91/01305 (**D5**) discloses a tandem construct comprising a "rainbow protein", a DNA/RNA or a peptide linker and an "energy transfer acceptor" or quencher, wherein it is said that this general "energy transfer acceptor" can be also a fluorescent protein from Aequorea (see page 5, lines 2-9 and page 6, line 20 to page 7 line 20). **D3** also refers to tandem fluorescent constructs comprising a fluorescent-labelled peptide conjugated with a non-peptide part. **D2** uses tandem fluorescent constructs, wherein both acceptor and donor pairs are not proteins. Thus, in view of this prior art, the IPEA considers that the subject matter of claims 46-47 does not fulfil the requirements of Articles 33 (2) and (3) PCT. Moreover, in the light of the cited prior art (see paragraph (b) above), claims 48-56 do not fulfil the requirements of Article 33 (3) PCT.

3. According to Article 6 PCT in combination with Rule 6.3 PCT, the claims must be clear and concise and they shall define the matter for which protection is sought in terms of technical features. Thus, the IPEA considers that :

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a) a protein, polypeptide, gene, DNA or nucleic acid, etc.. being chemical products must be characterized by a specific amino acid and/or nucleotide sequence. Thus, the use of arbitrary abbreviations without a clear technical meaning, i.e. "P4-3", "W7", etc.. in claims 3, 9, 18, 35, etc.. without any reference to their specific sequences, does not fulfil the requirements of Article 6 PCT in combination with Rule 6.3 PCT.

b) the use of the wording "about" in several claims is open to different interpretations and it only introduces unnecessary ambiguity to the actual scope of the claim.

c) the reference to a "cleavage recognition site having a random amino acid sequence" in claims 12 and 51 is ambiguous. Even if the cleavage activity of a protease has an unknown or partially defined specificity, the "cleavage site" has a specific amino acid sequence. In fact, it is the "linker moiety" which has a random amino acid sequence (see also page 25, lines 1-18 of the description).

d) the use of the wording "normally" in claim 23 is also ambiguous. Under certain conditions a cell can express a specific protease which is however not expressed under other conditions (temperature, pH, ionic strength, presence inducer, etc..). The skilled person would not know when said conditions should be considered "normal" or "not normal" (depending on cell type, etc..).

e) the reference to "lower than expected" in claim 27 is ambiguous as far as it is not clearly and explicitly said what has to be expected or at least how to determine what has to be expected (definition of a standard and/or control assay). In this respect, claims 36 and 41 also define the degree of fluorescence resonance energy transfer as related or reflecting to the amount of enzyme activity in the cell. However, this relation is not explicitly defined in the claims (low fluorescence indicates high presence of enzyme ? and low in respect to what ? ) (moreover in view of claim 37 which refers to "exogenous" enzyme. Presence of "endogenous" enzymes ??). Claim 40 also refers to a first and a second time but without specifying any further conditions (absence and presence of enzyme, before and after expression of the construct, etc. ?)

f) the reference to "**the**" cleavage recognition site in claim 33 is ambiguous as far as this cleavage recognition site is not clearly defined, i.e. "a cleavage recognition site

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specific for (said) protease".

**g)** the methods of claims 42-45 are broadly defined and they will not achieve the intended result. The compound to be tested can alter and/or modify : (a) the peptide linker moiety (unspecific binding, direct specific or unspecific cleavage, etc..), (b) the fluorescent protein moieties (unspecific binding, excitation and/or emission spectra, resonance energy transfer, etc..), (c) the conditions of cleavage (pH, ionic strength, etc..), and /or being also a fluorescent compound and/or a quencher compound, etc... but without altering the activity of an enzyme.

**h)** the dependency of claim 54 (on claim 51) is not correct (the subject matter of claim 52 is the one referring to a cell).